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REVIEW

WILEY

# A review of nicotine-containing electronic cigarettes—Trends in use, effects, contents, labelling accuracy and detection methods

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## Abstract

Electronic cigarettes (ECs) are thought to be less harmful than traditional combustible cigarettes and were originally intended to help smokers quit. Over the past two decades, they have especially gained popularity with the younger generation. To date, there are over 7000 unique e-liquid flavours available and over 400 different e-cigarette brands. The accuracy of nicotine strength labelling in e-liquids was assessed in this work. Twenty-three studies from around the world were chosen to assess the level and frequency of nicotine mislabelling in 545 e-liquid products. Nicotine strengths were most commonly mislabelled by between 5% and 20%, with the majority testing lower than what the label indicated. Fifteen European e-liquids that were assessed were labelled as 20 mg/ml or less, yet when tested, they contained more than 20 mg/ml of nicotine. One e-liquid that was supposed to contain no nicotine in fact contained 23.91 mg/ml of nicotine. Furthermore, the difference between the medians of the available labelled and experimental nicotine concentrations was significant ($p < 0.001$, Wilcoxon signed rank test). Preliminary studies show that high nicotine levels delivered via aerosol increase the risk for nicotine poisoning and cause airway inflammation. Other EC ingredients, such as flavourings, contribute to EVALI and 'popcorn lung'. There is evidence that certain flavourings, such as menthol, reinforce the effects of nicotine and modify drug absorption and metabolism. There is a global need for better quality control in EC products in order to make these safe for consumers.

**KEYWORDS**
electronic cigarettes, flavouring, labelling, nicotine, safety

## 1 | INTRODUCTION

Smoking is the leading cause of preventable death worldwide, accounting for 12% of all adult mortalities. Although tobacco use is steadily declining in most countries, electronic cigarettes (ECs) are increasingly gaining popularity.[1] The first ever EC, then called 'smokeless non-tobacco cigarette', was patented in 1965 by Herbert A. Gilbert. The aim of this cigarette was to reduce the absorption of harmful substances that are present in regular cigarettes.[2] Modern day e-cigarettes were first introduced on the market in China in 2004.

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These were developed and patented by pharmacist Hon Lik in 2003 as a safer alternative to smoking cigarettes.[3]

ECs are battery powered devices that deliver an aerosolized solution with or without nicotine. The solution is an e-liquid that is heated, aerosolized and inhaled. E-liquids generally contain propylene glycol (PG), vegetable glycerine (VG), water, nicotine and flavourings.[4] First generation e-cigarettes resemble traditional cigarettes. They are disposable or have a reloadable cartridge. Second generation e-cigarettes, known as vapes or vape pens, have a refillable reservoir for the e-liquid. In third generation e-cigarettes, known as mods or tank systems, the wattage and voltage can be modified. The term 'fourth generation e-cigarettes' encompasses the newest wave of e-cigarettes. A popular product in the fourth generation is JUUL.[5] It is categorized by prefilled cartridges termed 'pods', solutions with high nicotine concentrations and a sleek shape that allows the device to be used covertly.

E-cigarettes do not contain the typical carcinogens present in tobacco smoke although health hazards may still arise from solvents, flavours, additives and contaminants. It is debatable whether they help smokers quit or reduce cigarette consumption.[4,6] Using these products reportedly increases the risk of heart disease and lung disorders.[7] E-cigarettes are harmful to the user and nonusers who are exposed to the aerosol second hand and are most dangerous to children and adolescents.[8] Many e-cigarette users continue to smoke conventional tobacco products, which exposes them to higher levels of varying toxicants.[9] Assessing the safety of e-cigarettes and liquids is problematic due to the wide variety of devices and e-liquids sold, labels often being incomplete, and possibility for modification and personalization of e-liquids and devices. For example, users are able to modify the strength and throat hit from the vapour using different temperature settings on the device or dripping the e-liquid directly onto a heated coil.[10] Furthermore, many diseases, such as respiratory disease, take a long time to develop.[6,9]

E-cigarettes are evolving at a rapid rate, making it necessary to frequently adapt laws. By January 2014, just 10 years after the first EC was launched, there were 466 brands of e-cigarettes and 7764 unique e-liquid flavours. Internet sales were found to be substantial and largely unregulated.[11] Average nicotine concentrations in the United States e-cigarettes increased over time between 2013 and 2018 while there were considerable declines in traditional cigarette smoking.[12] Although nicotine is a legal drug, authors have advocated that it should still be regulated and monitored as it can be dangerous in certain quantities, especially for the younger generation.[12] The efficacy of nicotine delivery by e-cigarettes is not well understood and will vary due to several factors such as use characteristics and quality control.[13]

The aim of this review was to explore the authenticity and potential risks of use of nicotine-containing ECs due to the numerous reports of mislabelling and dangerous or unidentified ingredients. The main objectives were to (1) review and compile studies that have found labelling discrepancies and/or harmful ingredients; (2) determine whether these discrepancies fall within an agreed range (e.g. ±10% or ±30% as used in toxicology laboratories for validation studies) or are large enough for concern; (3) review detection methods of nicotine and other substances in ECs; (4) explore case studies where users have encountered health issues related to EC use; and (5) explore symptoms of EC users.

## 2 | METHODS

### 2.1 | Database search

There are many names for e-cigarette products such as EC, e-cig, electronic nicotine delivery system (ENDS), electronic nonnicotine delivery system (ENNDS), alternative nicotine delivery system (ANDS), personal vaporizers, e-hookahs, vape pens and vapes. For the sake of simplicity and limiting the number of articles, the initial search included the keywords 'electronic cigarette' and 'nicotine' as these are the terms most people use to describe EC products. In order to limit the number of hits and maintain relevance, these keywords were only searched for in article titles. Any studies before 2010 were excluded in order to represent more recent trends in mislabelling and also to represent more recent types of ENDS, which are more common and widespread. Figure 1 includes details of the search.

Further sources were obtained by consulting the reference lists of the chosen articles from Figure 1 and by consulting government websites. After reviewing the initial articles found it was clear that the use of flavourings in e-liquids was also a large concern, therefore a search was carried out on flavourings in e-cigarettes. The keywords used were 'electronic cigarette', 'e-cigarette', 'flavour' and 'flavor', and these were also limited to the article titles. Figure 2 details this search.

### 2.2 | Calculations and statistics

To assess mislabelling frequencies and nicotine laws around the world, 23 recent studies from the initial database search were chosen. The other 22 were excluded from calculations due to lack of clarity surrounding the units used for nicotine content. The following equation was used to calculate percentage difference between labelled and experimental nicotine levels in studies where these values were not given. Where the percentage values were given, it was determined that this was the equation that was used:

$$\frac{\text{Labelled} - \text{Experimental}}{\text{Labelled}} \text{x} 100.$$

Mislabelling frequency graphs and the e-cigarette law figure were generated using Microsoft Excel for Mac, version 16.35. To assess mislabelling discrepancy relevance, a Wilcoxon signed rank test was carried out with IBM SPSS Statistics for Mac, version 26.0, comparing labelled amounts of nicotine and actual quantified amounts of nicotine.

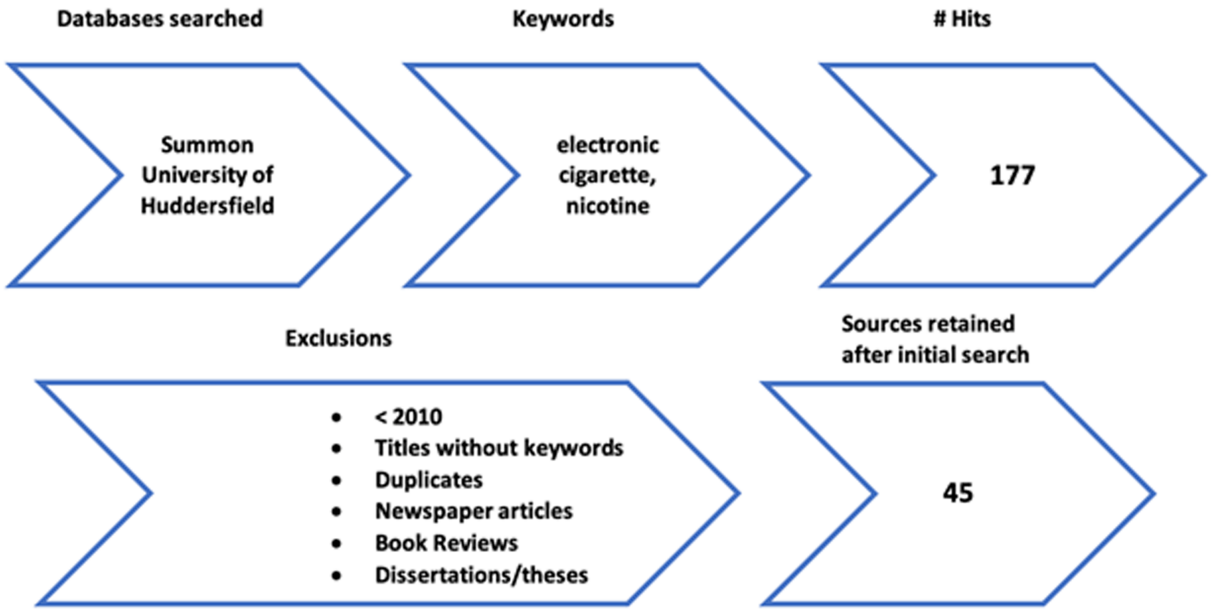


**FIGURE 1** Methods used for the initial search of articles

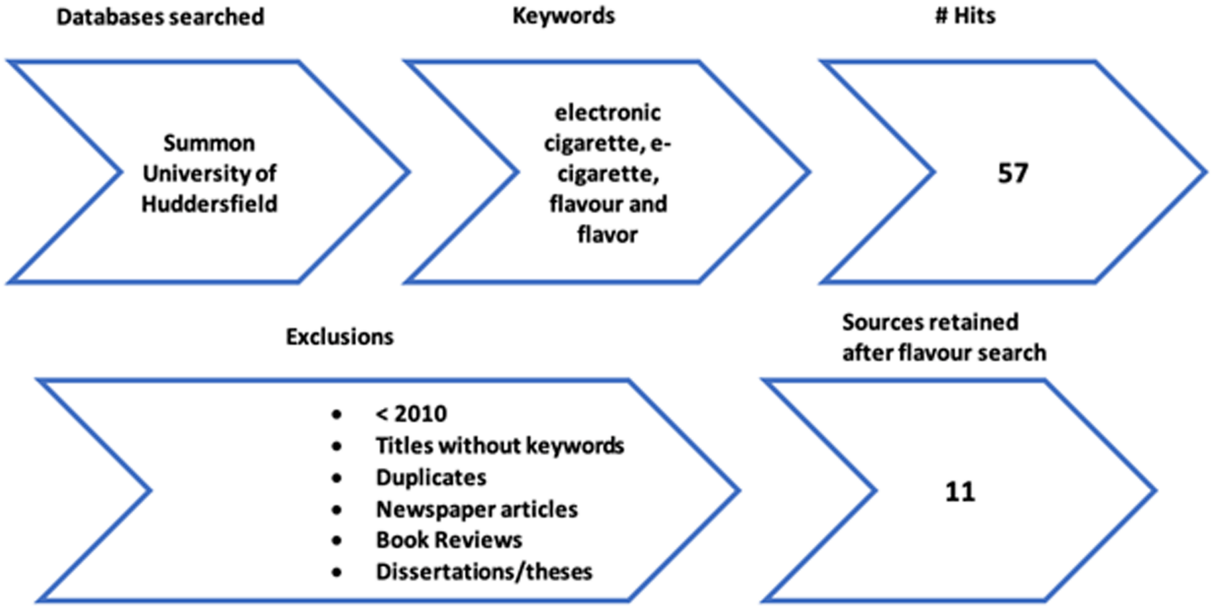


**FIGURE 2** Methods used for the search of articles on flavourings in e-cigarettes

## 3 | LAWS AND LEVELS AROUND THE WORLD

Up until 2014 there was a loophole that allowed e-cigarette products that contained nicotine to be sold without a medicines licence. Manufacturers and sellers were not obligated to mention whether their products contained nicotine or were free from harmful ingredients. The European Directive 2014/40/EU was introduced in 2014 to regulate the ingredients in e-cigarettes and include them in the category of tobacco related products.[14] This directive became applicable to all EU member states in 2016. The maximum threshold for nicotine content in refill liquids is 20 mg/ml in the United Kingdom and the EU. There are no medicinally licensed nicotine vaping products in the United Kingdom. In 2016, the FDA declared that ECs and flavoured e-cigarette liquids are tobacco products and are therefore subject to regulation that governs their manufacturing, marketing and distribution.[15]

To date, there is no nicotine cap for e-cigarettes in the United States; however, in late 2019, it was announced that a new legislation would be put in place to cap nicotine in e-cigarette products at 20 mg/ml.[16] There are laws on ECs for 89 countries. The sale of vapes is entirely banned in 30 countries and sales are regulated in

59 countries. There are only three countries that ban the sale of nicotine containing e-cigarettes: Australia, Japan and Jamaica. All other countries (106) do not have any regulations whatsoever for e-cigarettes or do not provide any data on the subject. Table 1 details the laws governing e-cigarette products in a selected few countries.

Only 45 countries out of the 89 that have laws surrounding ECs impose an age limit to buy and use these products, with 18 years being the most common age.[20] Alarmingly, e-cigarette flavourings design and marketing highly appeal to youths. In the United States, in 2014, youths used ENDS more than any other tobacco product. The FDA commissioner recently used the term epidemic to describe e-cigarette use in the United States. There is a growing concern that youths who use ENDS would not have consumed any other tobacco products had e-cigarettes not existed. Since 2014, e-cigarettes have been the most commonly used tobacco product among high school students. Interestingly, e-cigarette use declined in adults from 2015 to 2017. E-cigarettes have the potential to benefit adults if used as a complete replacement for combustible cigarettes; however, these products are not deemed safe for youths.[12] The flavoured e-liquids that appeal more to youths may be more dangerous than non-flavoured e-liquids since they contain more compounds that are potentially harmful when inhaled.[21] Figure 3 shows the countries where e-cigarettes are currently regulated to some degree (as of December 2020), even if this is only the age of purchase, the countries where ECs are entirely banned, the countries that have a ban on nicotine in EC products and the countries where ECs are unregulated.

Product regulation can include a number of factors and as mentioned earlier, some countries only regulate the age of purchase, not what the EC may contain or how it is marketed. The regulatory domains of ECs are minimum age to purchase, sale, advertising, promotion, packaging (child safety, health warning label), nicotine concentration, ingredients and flavours.[20] Even if a country has regulations imposed, this does not necessarily mean that EC products are safe in that country. For example, the United States regulates ECs in the sense that they are considered a tobacco product; however, there is no limit to the quantity of nicotine per e-cigarette or per cartridge.

## 4 | NICOTINE

### 4.1 | Pharmacokinetics and pharmacodynamics

Nicotine is a highly addictive psychoactive substance. It is an alkaloid obtained from the dried leaves of the tobacco plant and acts as a natural botanical insecticide. Nicotine is highly toxic to humans, even in small doses. The fatal dose is estimated at 30–60 mg for adults and around 10 mg for children. Fatal blood levels have been recorded as low as 115 μg/l in the relatively recently deceased (<136 h), although generally, fatal nicotine blood levels are seen at 5 mg/l and above.[22]

**TABLE 1** Laws governing e-cigarettes in various countries

| Country | Age Restriction | Nicotine Restriction | Regulations for Nicotine Containing Products | Regulations for Nonnicotine Products | Reference |
|---|---|---|---|---|---|
| UK | 18 | 20 mg/ml | TPD (2014/40/EU) translated to UK law through Tobacco and Related Products Regulations 2016 | General Products Safety Regulations 2005 | 17 |
| Australia | 18 | 0 mg/ml | Varies across states and territories but generally illegal to sell or buy nicotine for use in e-cigarettes, unless for a therapeutic reason or prescribed by a doctor<br>Only a 3-month supply of nicotine can be imported at a time | Regulated as smoking products under the Tobacco and Other Smoking Products Act 1998 | 9 |
| Canada | 18 | None | Vaping products subject to multiple acts<br>No upper nicotine limit, however, the concentration must be displayed along with the warning: 'Nicotine is highly addictive' | Vaping products are subject to multiple acts | 18 |
| USA | 21 | No federal regulation | American E-Liquid Manufacturing Standards Association (AEMSA) = volunteer organization have placed upper nicotine limit of 36 mg/ml with a ± 10% tolerance<br>In 2016, FDA required e-liquid manufacturers to register and apply for a licence to produce each flavour and nicotine concentration<br>Requires listing of each ingredient with its quantity and health effects | Since 2016, FDA requires e-liquid manufacturers to register and apply for a licence to produce each flavour and nicotine concentration<br>Requires listing of each ingredient with its quantity and health effects | 19 |

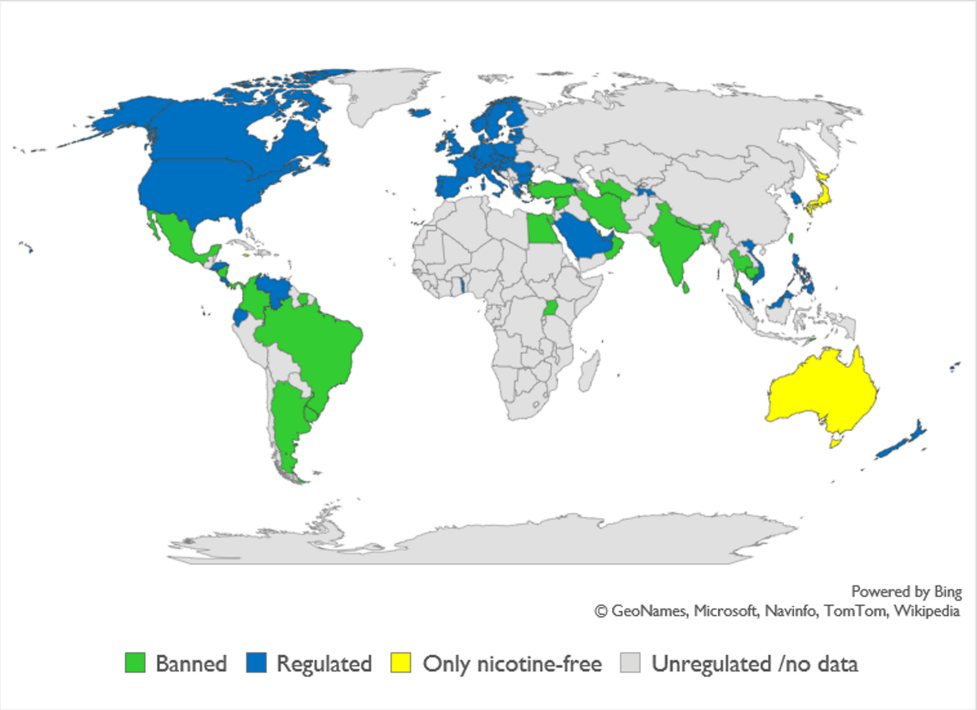


**FIGURE 3** World map of e-cigarette laws as of December 2020

Poisonings can occur from the ingestion or inhalation of nicotine as well as dermal contact with nicotine.[22] Nicotine is easily absorbed from the gastrointestinal tract, the buccal mucosa, the respiratory tract and the skin. It undergoes first pass metabolism when ingested, reducing its bioavailability. When nicotine is inhaled, it rapidly reaches the bloodstream where it is transported to the brain and binds with nicotinic acetylcholine receptors. Nicotine has a higher affinity for receptors in the brain and also binds to brain tissue more than any other part of the body. Binding capacity with brain tissue is increased in regular smokers. It has been shown that this phenomenon can lead to respiratory paralysis.[23] Nicotine is a weak base with a $pK_a$ of 8.0. In traditional smoking products, nicotine is unionized facilitating pulmonary absorption and transfer across membranes. Nicotine is metabolized into an extensive number of metabolites by the liver. The primary pathway used is via cytochrome P450 isozyme CYP2A6. The main metabolic pathways of nicotine in the body are shown in Figure 4. Approximately 5% of nicotine is excreted unchanged in the urine within 24 h while 10% is excreted as cotinine, which is the main marker used to detect nicotine use. Smoking nicotine also has secondary effects on metabolism, such as metabolism of other drugs, particularly those metabolized by CYP450 and CYP1A2.[24]

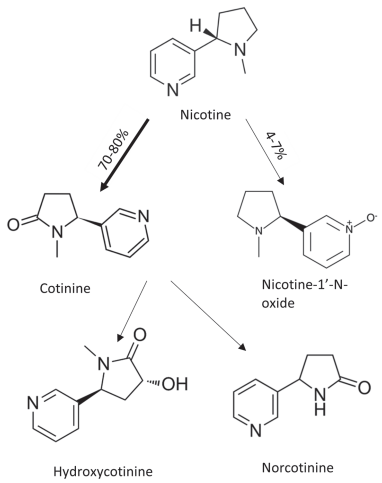


**FIGURE 4** Main metabolic pathways of nicotine

## 4.2 | Nicotine salts

In nature, nicotine is in its salt form. The ionized form of nicotine does not readily cross organic membranes. Nicotine in traditional cigarettes and e-cigarettes is in its free-base form allowing it to cross membranes more freely, thus making it more bioavailable. The free-base form will also vaporize more easily, making it ideal for cigarettes and e-cigarettes. Nicotine salts for e-cigarettes are formed by adding an acid, usually benzoic acid, to the natural nicotine salt. This helps the salt vaporize at lower temperatures, reduce the pH for a smoother 'throat hit' and be absorbed better by the body.[24] Comparisons between nicotine salts and free-base nicotine are shown in Table 2.

The reason for the recent explosion in the popularity of nicotine salts is that users are constantly looking for higher and higher nicotine concentrations, however, with higher nicotine comes considerable discomfort when vaping. JUUL pods, among other brands, contain dissolved nicotine salts. This allows a higher level of nicotine to be inhaled more easily and with less irritation than free-base nicotine in traditional e-cigarettes and other tobacco products.[12] Users also

**TABLE 2** Nicotine salts vs free-base nicotine (adapted from VapeUK[25])

| Nicotine Salts (Prepared) | Free-Base Nicotine |
|---|---|
| Contains benzoic acid or citric acid added | Deprotonated with ammonia |
| Vaporizes at lower temperatures | Requires higher temperatures to be vaporized |
| Less volatile | More volatile |
| No smoke cloud or small amount of smoke, more discreet | Enables large cloud production |
| Suited to lower power devices | Requires higher power devices for maximum vapourizing |
| Suited to high nicotine strengths | Suited to low-medium nicotine strengths |
| Slow oxidation means longer shelf life | Faster oxidation means shorter shelf life |
| Cannot handle high flavour complexity | Can handle high flavour complexity |
| Less liquid needed for comparable nicotine hit | More liquid needed for comparable nicotine hit |
| Smooth at high doses | Harsh at high doses |
| Fast absorption into the bloodstream | Slower absorption into the bloodstream |
| More nicotine absorbed into the bloodstream | Less nicotine absorbed into the bloodstream (excess is breathed out in vapour cloud) |

report having an instant rush of nicotine when smoking the salts compared to free-base nicotine. Nicotine salts are less volatile; therefore, a greater fraction of the nicotine in the e-liquid is absorbed by the body rather than lost in the exhaled vapour. This form of nicotine is also much more rapidly absorbed by the body.[24]

JUUL is a brand that uses nicotine salts and this brand is most commonly used by youths. Several studies have reported that JUUL pods already contain a higher level of nicotine than in regular ECs. These studies have reported JUUL pods to contain between 56 and 75 mg/ml nicotine, this is almost four times the regulated amount of nicotine allowed in other countries.[26] This is a very high level of nicotine for young people to be consuming on a regular basis—young people who have not had a lifelong addiction to nicotine and have not been exposed to it through years of conventional cigarette smoking. Furthermore, nicotine salts are more attractive to youths because they are less volatile and produce less smoke, allowing their smoking to be more discreet.[11]

## 5 | MISLABELLING OF E-CIGARETTES

Mislabelling of nicotine or failure to state the nicotine level on e-liquids is dangerous for many reasons. Nicotine is highly addictive and toxic even in small doses. A mislabelled low nicotine e-liquid may put an inexperienced user at risk of overdosing as this may mislead them to consume more believing it is safe.[27] For example, one study determined the nicotine concentration in one 5 ml e-liquid vial to be 20 mg/ml. This whole vial contained 100 mg total nicotine. Another study found as much as 720 mg of nicotine in one bottle of e-liquid, highlighting the issue of selling large bottles and quantities of e-liquids.[28] As mentioned earlier, 30–60 mg of nicotine is lethal for adults and 10 mg is lethal for children.[29] This is dangerous if large quantities are smoked within a small timeframe, the e-liquid is diverted for a use other than intended or poisoning from ingestion.

### 5.1 | Mislabelling in nicotine-containing e-liquids

The frequency of nicotine mislabelling was determined from 23 different studies conducted between 2013 and 2020. The frequency of mislabelling was divided into 12 percentage ranges showing the difference between labelled nicotine and what was actually quantified (Figure 5). The most common occurrence of

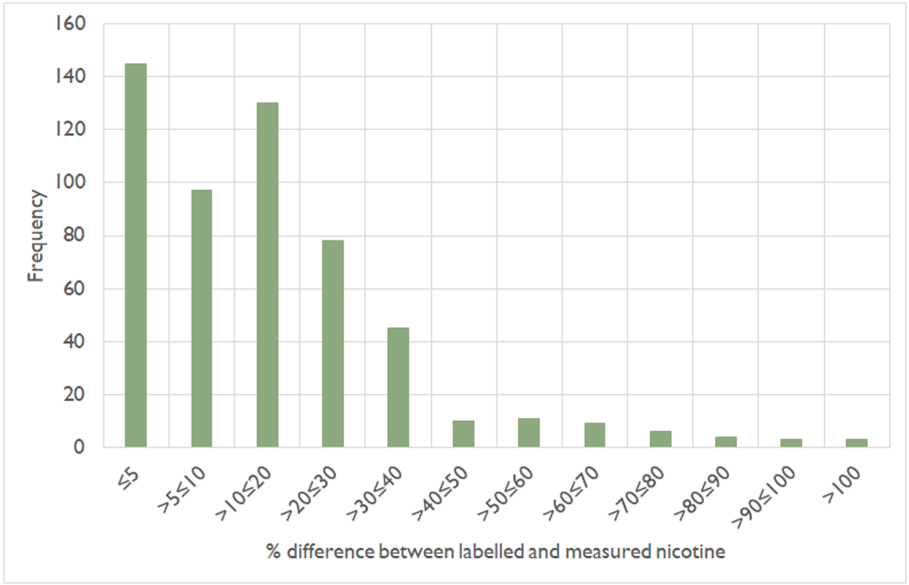


**FIGURE 5** Nicotine mislabelling frequency in e-cigarette products according to % nicotine difference between labelled and measured content. The data were collated from 23 studies published between 2013 and 2020 and excludes nicotine-free products

mislabelling was between 0% and 5% deviation from the labelled nicotine according to the 23 studies used. AEMSA and the British Standards Institution (BSI) have imposed a tolerance limit of ±10% deviation from the labelled amount and many countries have adopted this value also.[19,30] The frequency of 0%–5% mislabelling should not then be alarming. However, the second largest frequency of mislabelling was in the 10%–20% range.

It is important to note that theses 23 studies were conducted in different countries that have different laws and different quality control regulations, should they exist at all. It is also important to note that these studies tested different e-liquid and EC products in varying quantities and varying nicotine concentrations and used different analytical methods. Details of all 23 studies are shown in Table 3.

There were several specimens that were excluded from this chart. Some of the labels simply indicated 'high', 'medium' or 'low' nicotine. In these cases, the studies tried to estimate the nicotine levels; however, this does not give a clear indication as to what the nicotine concentration should have been and therefore the percentage difference between measured and labelled could not be determined.

There were two occasions where the label indicated that the e-liquid contained nicotine (3 mg/ml) and when tested it did not, hence the 100% difference between labelled and measured nicotine. This could be due to no nicotine being present or the level of nicotine in the product was below the limit of detection or quantification.[48] In one case, there was a 103.3% difference between the labelled and quantified nicotine; however, this indicated that the quantified nicotine was more than double the labelled nicotine, neither of which were zero. Out of the 545 e-liquid samples from the 23 studies, 107 contained nicotine at a level above 20 mg/ml; however, many of these studies were conducted in the United States or samples were obtained from the U.S. market where there is no legal upper limit for nicotine concentration in e-liquids. Of the 107 e-liquids that contained more than 20 mg/ml of nicotine, 15 were from countries that have imposed a 20 mg/ml nicotine limit: UK, Greece, France and Poland. All but two samples were purchased before the TPD became applicable in 2016; therefore, e-liquids containing more than 20 mg/ml were not yet illegal. The two samples that contained

**TABLE 3** Summary of studies that detected nicotine labelling inaccuracies from 2013 to 2020

| Reference | Labelled Nicotine, mg/ml | Quantified Nicotine, mg/ml | Difference With Label | Method | Country |
|---|---|---|---|---|---|
| [28a] | 12 to 30 | 11.7 to 25.7 | −15.42% to +21.1% | UHPLC | Switzerland, France, UK and USA |
| [31] | 4 to 24 | 2 to 25 | −89% to +28% | GC-TSD | Poland, UK and USA |
| [29] | Low to 24 | 8.5 to 22.2 | −20.4% to −7.5% | LC-MS | USA |
| [32] | 6 to 60 | 5.6 to 72.9 | −12.9% to +89% | HPLC | USA |
| [33] | 8 to 100 | 7.4 to 97.7 | −58.89% to −2.3% | GC-MS | USA |
| [34] | 12 to 24 | 9.5 to 25.8 | −21% to +22.1% | GC-FID | Greece, USA, UK and Italy |
| [35] | 5 to 210 | 1.2 to 150.3 | −92.5% to +103.3% | GC-NPD | USA, South Korea and Poland |
| [36] | 3 to 18 | 3.1 to 17.5 | −32.2% to +3.3% | GC-TSD | South Korea, USA, Italy, Netherlands and China |
| [37a] | 12 to 25 | 12.9 to 25.8 | −15.5% to 10.6% | GC-TSD | Poland |
| [38] | 6 to 24 | 3.3 to 20.5 | −38% to +3.75% | GC-MS/MS | USA |
| [39] | 6 to 24 | 4.98 to 19.3 | −49.17% to −1.16% | GC-MS | USA |
| [30] | 1.8 to 18 | 1.2 to 18.6 | −33.3% to +10% | LC-MS/MS | Italy, China, France Italy, USA and UK |
| [40] | 16 to 24 | 11.2 to 24.2 | −48.75% to +0.83% | GC-NPD | USA |
| [31] | 6 to 22 | 4.3 to 14.7 | −55% to +39% | HPCL-MS/MS | USA |
| [41a] | 16 to 48 | 15.5 to 50.1 | −12.78% to +28.34% | GC-MS/FID | Switzerland |
| [13] | 6 to 36 | 6.26 to 37.22 | −2.94% to +25.2% | FT-ICR-MS | USA |
| [42] | 3 to 18 | 3.36 to 20.86 | +12% to +17.9% | HPLC | New Zealand |
| [43] | 8.65 to 15.9 | 6.76 to 16.3 | −24.7% to +2.5% | GC-FID | South Korea |
| [19] | 18 | 11.6 to 27.4 | −35.3% to +52.4% | HPLC | USA |
| [44b] | Nicotine-free | 0.5 to 2.9 | +>100% | GC-MS | Australia |
| [27] | 6 to 24 | 5.4 to 24.3 | −16.7% to +30% | GC-MS | Macedonia |
| [45] | 6 to 12 | 0.22 to 17.3 | −96.3% to +76.9% | GC-MS | Malaysia |
| [46b] | 3 to 24 | 0 to 20.5 | −>100% to −0.91% | HPLC | Morocco, USA, France, Germany, Spain, UK, Belgium and China |
| [47] | 3 to 6 | 1.88 to 5.61 | −37.34% to +12.34% | LC-MS/MS | USA |

[a]These studies claimed that the nicotine content did not deviate much from the label and the levels found were acceptable.
[b]+>100 or −>100% indicate a change from a positive to a null value or a null value to a positive value, respectively.

more than 20 mg/ml were labelled as 45 mg/ml and were purchased from a U.K.-based website in 2017.[49]

Although the majority of mislabelling was found to be in the 0–5% range, this could still be dangerous for users that can modify the voltage on their e-cigarette products. Several studies have shown that by increasing the voltage, a higher concentration of nicotine is transferred into the aerosol. It has been shown that these increases are not linear. It cannot be predicted exactly how much nicotine will be transferred into the aerosol by using a specific voltage.[41]

To establish whether there was a statistically significant difference between the labelled and analysed nicotine concentrations, a Wilcoxon Signed rank test was performed. The distribution of the data sets was analysed using histograms to determine whether the data were normal. Some data sets appeared evenly distributed whereas others did not. Nonnormal distribution was confirmed by the Kolmogorov-Smirnov and Shapiro-Wilk tests for normality ($p < 0.05$). Therefore, it was determined that a nonparametric test was most appropriate to use here. Two previous studies that compared labelled and experimental nicotine concentrations in e-liquids used a paired *t* test.[46,48] As the data in this study are not normally distributed, the nonparametric equivalent of a paired *t* test was used. The Wilcoxon signed rank test indicated that the difference between the medians of labelled and experimental nicotine concentrations was significant ($p < 0.001$). As with Figure 5, the values used in these statistics tests excluded labels where it was not explicitly stated what the nicotine level was.

## 5.2 | Mislabelling of nicotine-free e-liquids

Nicotine-free products that do in fact contain nicotine will put users at risk of developing addiction or not being able to reduce their nicotine consumption effectively. This is also dangerous for people with nicotine allergies and pregnant women who vape nicotine-free products.[50] There were several studies where nicotine was found in supposedly nicotine-free products. One study found as much as 23.91 mg/ml nicotine in a 'nicotine-free' labelled e-liquid.[19] Figure 6 shows, where possible, the EC manufacturer name/brand and the average amount of nicotine found in their 'nicotine-free' labelled products. As mentioned for Figure 5, the studies shown in Figure 6 all used different e-cigarette products and tested a different number of samples.

As well as the issue of mislabelling, there is also no guarantee that the composition of e-liquids will be constant across batches, especially in the absence of guidelines and standards, which is the case in many countries where these are not yet mandatory.[49] Several studies have shown differences in quality between brands but also alarmingly within the same brands. This means that any studies that have claimed to have found that the nicotine concentration was accurate and corresponded to the level indicated on the label may be false for an e-liquid of the same brand belonging to a different batch.[27,48]

Not only is mislabelling an issue, but the type of label is also an issue. Some brands use numbers without representative units (e.g., 18 mg or 18). This causes confusion for the consumer as they do not know whether the whole e-liquid vial contains 18 mg of nicotine or whether it contains 18 mg/ml.[51] Some brands do not even indicate nicotine concentration with a number; they simply label 'high', 'medium' or 'low', which could cover a wide spectrum of nicotine concentrations.[29]

Mislabelling is an even more disturbing issue when e-liquids that contain nicotine are sold and found in countries that have a ban on nicotine containing e-liquids. Due to this ban, the e-liquids will not be quality controlled and are at even higher risk of being largely mislabelled due to lack of government guidelines. This was the case in a study from Ontario, Canada, where a quarter of all products tested fell outside of a 10% threshold of their labelled nicotine concentration.[34] However, this study also found that the proportion of products with labelling discrepancies was much lower than in other countries including the United States, the United Kingdom, Poland, France, Switzerland, Greece and South Korea. Since these countries are allowed to sell nicotine-containing e-liquids, then it is more likely that there will be a wider variety of products with a higher chance of mislabelling to occur.

One study found that some manufacturers use cured tobacco leaves to extract the tobacco flavouring instead of using an industry-

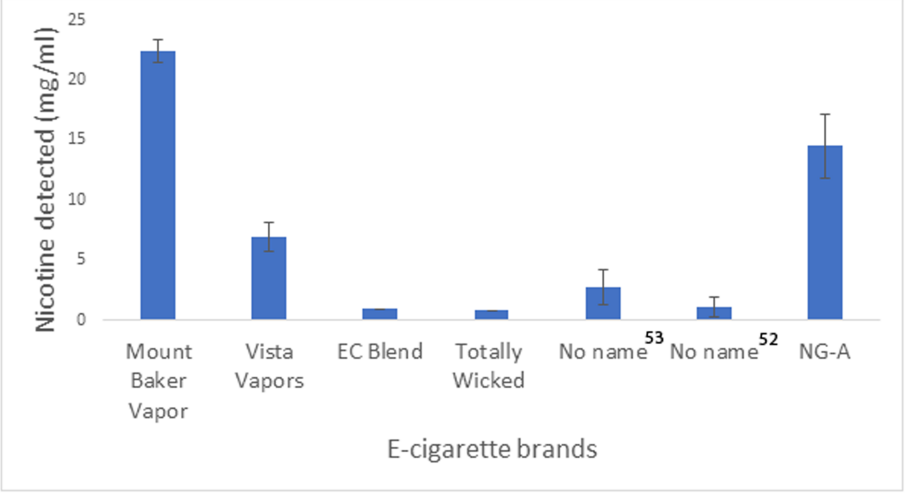


**FIGURE 6** Average nicotine detected in ng/ml in 'nicotine-free' labelled products including standard deviations. Where no brand name was given, references are provided to the relevant articles

produced flavouring. This leads to the presence of tobacco-derived toxins in the e-liquids which may be higher than in conventionally produced e-liquids. In this scenario, significant deviation of the nicotine concentration from the labelling will inevitably lead to inaccuracies in the levels of tobacco-derived toxins also.[46] Tobacco specific nitrosamines (TSNAs) are associated with negative health effects in traditional tobacco cigarettes due to their abundance and carcinogenicity. TSNAs are not specifically labelled; however, some e-liquids have 'contains known carcinogens' labelled. If the nicotine levels are mislabelled in these types of e-liquids, then the TSNAs will also be higher or lower than predicted and this could lead to serious health issues.[46]

Although the majority of the mislabelled samples actually contained less nicotine than advertised, this still poses a health threat. A recent study found that users commonly show compensatory behaviours when vaping low nicotine products. Users demonstrate more intense puffing and increased power settings in an attempt to deliver more nicotine. As a result, the user is exposed to higher concentrations of other chemicals in the EC such as formaldehyde.[47,52]

## 6 | OTHER INGREDIENTS AND COMPOUNDS IN E-LIQUIDS

### 6.1 | Tobacco alkaloids and tobacco-specific nitrosamines

High nicotine content and nicotine mislabelling are not the only worrisome aspects of e-cigarettes. Nicotine used in the production of e-liquids is extracted from the tobacco plant. As a result, tobacco-specific impurities may be present in the final product. These are tobacco minor alkaloids: nornicotine (NN), anatabine (AT), anabasine (AB) and myosmine (MS) and cause the formation of degradation products such as cotinine (CT), nicotine-*N* ′-oxides (NO) and β-nicotyrine (BN). These degradation compounds can also originate from inadequate handling and storage.[30,48,53]

Tobacco may also be used to obtain the tobacco flavour without the nicotine content. These flavours are obtained by curing tobacco leaves and performing a solvent extraction. This produces natural extract of tobacco (NET) liquids. Although the nicotine is removed through this process, tobacco specific nitrosamines (TSNAs) often remain. These include nitrates found naturally in the tobacco plant, phenols from heating the tobacco and aldehydes which can be present in the plant and also derived from heating the tobacco. TSNAs are known carcinogens. Industrially produced tobacco absolute is used to imitate the tobacco flavour without using the tobacco plant. The use of this artificial flavour may remove the TSNAs that are present when using actual tobacco plants.[46]

### 6.2 | Flavourings

Although ENDS are thought to be generally safer than traditional tobacco cigarettes, because they contain fewer toxicants and carcinogens in the aerosol, there is a vast array of flavourings that are used in EC products that lack clinical and toxicological studies. Due to this and rapid market growth, regulatory action is hindered. To date, there are more than 7000 unique flavourings and 400 brands of e-cigarettes.[53] Many types of flavouring are used in e-liquids that are generally recognized as safe (GRAS) flavour compounds for use in foodstuffs. However, health hazards from exposure to these chemicals through inhalation have not been assessed as GRAS only applies to ingestion. While many of the flavourings used in ECs are known respiratory irritants and toxicants, there is a lack of knowledge on the effects of long-term regular exposure to these chemicals.[40]

Diacetyl is a flavouring compound commonly used in food products that is GRAS. However, in the early 2000s, there were several reports of serious lung disease in microwave popcorn workers, hence the name 'popcorn lung'. Many flavouring compounds, when heated will transform into harmful chemicals. Flavourings are often not included on the ingredients list.[8] The reason these flavourings and ingredients are potentially very harmful to e-cigarette users is that these compounds are absorbed through the lungs and may damage pulmonary tissue and the cardiovascular system. When these GRAS flavourings are ingested, they bypass the pulmonary and cardiovascular system.[54] Many flavour chemicals are known to transfer efficiently into the aerosol. Several of these chemicals cause cytotoxicity and the higher the voltage, the more toxic to human lung cells, as more of the chemicals are transferred into the aerosols and are inhaled.[55] This is because the aerosol produces ultra-fine particles that easily reach the distal parts of the lungs and alveoli, resulting in efficient transmission of harmful chemicals to the lungs and bloodstream.[56]

Table 4 summarizes potentially harmful ingredients found in e-liquids in several studies. Although laws vary from country to country, some of these compounds that are banned have still been found in e-liquids. There are many other flavouring compounds that are potentially dangerous for health when aerosolized and inhaled; however, the most common ones found in the literature are presented here. It is also important to note that these flavourings and chemicals may react with one another and form secondary products that may be even more harmful.

#### 6.2.1 | Aldehydes

Until recently, it was thought that toxic aldehyde formation in e-liquids was due to thermal breakdown of propylene glycol (PG) and vegetable glycerol (VG). However, more recent evidence has shown that most aldehydes are a direct result of the thermal decomposition of flavouring compounds.[59] Breakdown of PG and VG still produces aldehydes but to a much lesser extent than flavourings. Although formaldehyde, acetaldehyde and acrolein are formed when the sugars in the e-liquids break down due to heating, these compounds have been found in unheated e-liquids. It is important to quantify levels of sugars (glucose, sucrose and fructose) in e-liquids to determine how much formaldehyde, acetaldehyde and acrolein the user would potentially be exposed to as aldehydes are known to cause respiratory

**TABLE 4** Potentially harmful ingredients found in e-cigarette liquids and their health effects

| Type | Reference | Ingredient | Hazard statement | Use | Health effects |
|---|---|---|---|---|---|
| *Aldehydes* | 15 | Formaldehyde | HPHC list (FDA) | N/A | Carcinogenic; respiratory toxicant |
| | 15 | Acetaldehyde | HPHC list (FDA) | N/A | Carcinogenic; respiratory toxicant and depressant; addictive |
| | 15 | Acrolein | HPHC list (FDA) | N/A | Eye and respiratory irritant; respiratory and cardiovascular toxicant |
| | 15,57 | Vanillin | Warning (ECHA) | Vanilla flavours | Respiratory irritant; eye irritant; can interact with nicotine to affect epithelial cell function |
| | 1,54,58 | Cinnamaldehyde | Warning; Harmful to aquatic life with long lasting effects (ECHA) | Tobacco, fruit, sweet and cinnamon flavours | Cytotoxic; genotoxic; eye irritant; skin irritant; may impair homeostasis in respiratory system |
| | 37,54,55,57–59 | Benzaldehyde | Warning; Harmful to aquatic life with long lasting effects (ECHA) | Cherry flavours | Cytotoxic; genotoxic; carcinogenic; respiratory irritant; harmful if swallowed; skin irritant; causes serious eye damage; causes damage to organs through prolonged or repeated exposure |
| *Ketones* | 8,14 | Diacetyl (2,3-butanedione) and structurally related compounds | Danger; Highly flammable liquid and vapour (ECHA) | Buttery, caramel, butterscotch, piña colada and strawberry flavours | Respiratory toxicant; harmful if swallowed; skin irritant; causes serious eye damage; causes damage to organs through prolonged or repeated exposure; associated with 'popcorn lung' disease and bronchiolitis obliterans (irreversible loss of pulmonary function) |
| | 8 | Acetoin or 3-hydroxybutanone | Warning; Flammable liquid and vapour (ECHA) | Buttery and caramel flavours | Causes serious eye damage |
| *Alcohols* | 54,60 | Linalool | Warning (ECHA) | Citrus flavours | Causes skin irritation; causes serious eye irritation |
| | 60 | Menthol | Warning (ECHA) | Mint flavours | Causes skin irritation; causes serious eye irritation |
| *Others* | 14,54 | Estragole | Warning; Harmful to aquatic life with long lasting effects (ECHA) | Anise flavours | Harmful if swallowed; eye irritant; skin irritant; carcinogenic; genotoxic; mutagenic; reprotoxic |
| | 35,55 | Methyl eugenol or eugenol | Warning; Toxic to aquatic life, (ECHA) | Clove and cinnamon flavours | Genotoxic; carcinogenic; respiratory toxicant<br>Possibility of pulmonary aspiration due to diminution of gag reflex due to local anaesthetic effects |

(Continues)

**TABLE 4** (Continued)

| Type | Reference | Ingredient | Hazard statement | Use | Health effects |
|---|---|---|---|---|---|
| | 54,60 | Limonene | Warning; Very toxic to aquatic life with long lasting effects; Flammable liquid and vapour (ECHA) | Citrus flavours | Eye irritant; skin irritant; may be fatal if swallowed and enters airways |
| | 26,60 | Ethyl maltol | Warning (ECHA) | Sweetener, cotton candy flavours | Harmful if swallowed |

irritation.[15,58]Aliphatic aldehydes are used for fruity flavours and aromatic aldehydes are used for sweet and spicy flavours. The Flavoring and Extract Manufacturers Association (FEMA) of the United States has identified over 1000 GRAS flavouring compounds as hazardous for respiratory health due to volatility and irritant properties; however, it is unclear whether these flavourings have been taken of the GRAS list.[1]

Cinnamaldehyde, a flavouring used to impart a cinnamon flavour, has been found to be cytotoxic in adult human pulmonary fibroblasts.[58] This flavouring compound was found in 20 out of 39 refill fluids tested in a 2016 study at concentrations ranging from 2.2 to 140,000 μg/ml. All of the concentrations were higher than the lowest observed adverse effect level. This study determined that cinnamaldehyde is one of the most cytotoxic e-liquid flavourings. This flavouring was even found in e-liquids where it was not expected as well as in higher doses in random flavours compared to actual cinnamon flavour e-liquids. Cinnamaldehyde aerosols were also more potent when made at a higher voltage. This is a dangerous issue for those who smoke DIY e-cigarette where the voltage can be modified. A study from 2017 also found cinnamaldehyde in all tested e-liquids and these exhibited dose-dependent immunosuppressive effects. Cinnamaldehyde has the potential to impair respiratory immune cell function.[1]

Benzaldehyde is a respiratory irritant which was detected in 108 out of 145 e-liquids in a 2016 study. It was most commonly associated with cherry-flavoured products, with yields between 5.13 and 141.2 μg/30 puffs. These levels are more than 1000 times lower than the permissible exposure limit in the e-liquid manufacturing workplace. This is concerning for workers who are periodically exposed to this chemical over long periods of time.[61]

### 6.2.2 | Ketones

As mentioned earlier, diacetyl is known to cause 'popcorn lung'. Diacetyl is a ketone used to impart a buttery flavour. Not only is there evidence of health issues related to mislabelling and related to certain flavourings, there are also synergistic effects when certain compounds are present in the same e-liquid. Acetoin has been used as a safer alternative to diacetyl; however, acetoin is a precursor to diacetyl. Diacetyl will form even when the e-liquid in left in storage, let alone when acetoin is aerosolized. Furthermore, the formation of diacetyl from acetoin is accelerated in the presence of nicotine.[62] A 2016 study found either diacetyl or acetoin in more than 90% of the tested e-liquids. Forty-six out of the 51 samples tested contained acetoin, up to 529 μg per e-cigarette and 39 contained diacetyl, up to 239 μg per e-cigarette.[8]

FEMA released a report in April 2012 that highlighted the risks associated with inhaling diacetyl along with other food flavouring chemicals. The warning mentions that heating and inhaling fumes of diacetyl may cause severe adverse health effects.[8] This warning was only found within the food production industry and not with e-cigarette manufacturers. Moreover, there are no health-based standards for diacetyl inhalation for children or the general public. Inhalation exposure limits to diacetyl and other food-flavouring compounds have been established for adult workers only.[8]

### 6.2.3 | Alcohols

Some e-cigarettes are reported to contain alcohol. Ethanol may be used as a solvent to dissolve flavouring particles; however, this ingredient is often not reported on the labelling. Inhaling alcohol is known to have toxic effects on the brain.[10] Ethylene glycol and diethylene glycol were detected and qualified but not quantified with GC-MS in e-liquids. These were said to be degradation products of propylene glycol.[28] Menthol is a common flavouring in e-liquids and traditional cigarettes. Its properties include cooling and local anaesthesia which may make the smoking experience more pleasurable. Menthol has been found to cause skin irritation and serious eye irritation. Moreover, there is evidence that menthol reinforces the effects of nicotine and has effects on drug absorption and metabolism.[60]

### 6.2.4 | Others

One study found caffeine in several flavoured e-liquids. These were chocolate, coffee, tea and energy drink flavoured e-liquids. The levels of caffeine found were significantly lower than the amounts usually found in caffeinated beverages and foods, which would remove any concerns; however, little is known on the effects of caffeine aerosolization and inhalation. Moreover, caffeine could have synergistic

effects when paired with nicotine and other components of e-liquids.[63]

In addition to these potentially harmful flavourings, many manufacturers sell do-it-yourself kits which allow the consumers to mix their own flavourings and nicotine concentrations. To the inexperienced user, this could be extremely dangerous.[54] Several studies have also determined device power to be a significant factor contributing to the toxicity of ENDS, especially when e-liquids are heated to high temperatures as this increases aldehyde reactivity.[40,55,56] Although single flavouring chemicals may be toxic, combinations of chemicals and voltage used contribute to overall toxicity of ENDS due to increased release of toxic carbonyl compounds.[40,61]

One 2019 study identified 59 flavour chemicals in JUUL flavour pods. Their concentrations varied from 0.01 to 16.7 mg/ml. The flavourings with the highest concentrations were menthol, vanillin and ethyl maltol. Cytotoxicity was assayed and was similar for nicotine with and without flavour chemicals. Cytotoxicity was much lower for flavour compounds alone, although cytotoxic effects were seen in all e-liquid flavours at concentrations of 1 mg/ml and above. Toxicity could be linked more to the high nicotine concentrations (average 60.9 mg/ml) associated with JUUL products or synergistic effects of flavourings and high nicotine content together.[26]

The Food and Drug Administration (FDA) has established a list of harmful and potentially harmful constituents (HPHC) in tobacco products and tobacco smoke. The European Chemicals Agency (ECHA) has 'infocards' of chemical compounds that are harmful or potentially harmful, but not necessarily related to EC products. Although these lists are established, not all chemicals mentioned are actually banned in EC products. According to the TPD, only vitamins, caffeine, taurine, colourants or additives that have carcinogenic, mutagenic or toxic to reproduction (CMR) properties in the unburnt form are prohibited in ECs. There also appears to be a lack of studies that describe the presence of illicit substances such as THC and other cannabinoids in advertised licit U.K. EC products,[64] indicating that these products are not being used by manufacturers as vehicles for delivering illicit substances covertly to users. It appears that there may not be a benefit for manufacturers to add cannabinoids and other substances to products that are legal, and the reverse has not been reported either (i.e., unexpected nicotine contents have not been reported in cannabinoid products).

## 7 | TOXICOLOGICAL EFFECTS OF E-CIGARETTES

E-cigarettes may be useful in helping smoking cessation in long term cigarette smokers; however, ECs are most commonly used by younger people who have never smoked a conventional cigarette or who were not regular smokers before using ECs. Due to the many available EC products, differences in engineering, components, ingredients and personalization/DIY potential, it is difficult to assess potential dangers and the exact sources of these. There is currently no conclusive information on the respiratory health effects of long term vape usage. Although studies have been carried out on human bronchial epithelial cells in vitro, further research is needed to assess the dangers on human respiratory health and overall health.[26,56]

One of the main concerns is that current e-cigarette batteries are large and last a long time. This allows the user to continuously smoke, unlike smoking a conventional cigarette which has a natural end. The reservoirs for e-liquids can also be large, allowing the user to smoke a significant amount of e-liquid in one sitting, exposing them to very high levels of nicotine.[10] Another concern is that the batteries in ECs can heat an e-liquid up to 350°C. Ingredients in the e-liquid may become modified at these high temperatures and produce dangerous or even carcinogenic compounds that are then inhaled. This is particularly concerning for pods, such as JUUL pods, that use nicotine salts, which already vaporize at lower temperatures and which produce rapid increases in brain nicotine levels.[10]

Since ECs have been available on the market, there have been reports of health issues and symptoms directly related to e-cigarette use. It is not clear what part of the EC causes each symptom and health condition; however, a few studies have attempted to pinpoint this. Exogenous lipoid pneumonia has been reported and directly linked to e-cigarette use. The source of this condition was recurrent exposure to glycerine-based oils. VG is present in most, if not all e-liquid formulations. The purpose of this ingredient is to produce the visual smoke when the e-liquid is aerosolized.[65]

PG and VG are the base ingredients for almost all e-liquids. There will be at least one of these in any e-liquid formulation. These ingredients have been declared safe for consumption; however, there is little information of long-term health effects from inhalation.[5] An extensive study conducted in 2013 determined that aerosolized propylene glycol and glycerol produce mouth and throat irritation and a dry cough.[66] As mentioned earlier, recent studies have shown that these compounds degrade into aldehydes when heated, however, to a much lesser extent than certain flavourings. These studies have also found aldehydes in unheated e-liquids.[15] One study conducted on rats determined that PG and VG aerosols showed limited biological effects and limited toxicity, whereas PG and VG with nicotine aerosols resulted in toxic lung and metabolic effects.[67]

### 7.1 | E-cigarette or vaping product use-associated lung injury

E-cigarette or vaping product use-associated lung injury (EVALI) has been reported frequently in the recent literature. The Centers for Disease Control (CDC) in the United States declared EVALI a national outbreak due to high incidence in 2019; 2500 people were hospitalized in the United States alone. Initial symptoms of EVALI include cough, shortness of breath, chest pain, nausea, vomiting, diarrhoea, fatigue, fever and weight loss. Patients have

progressively developed lipoid pneumonia, mainly associated with vitamin E acetate (VEA), eosinophilic pneumonia and chemical damage to the lung tissue. It appears that most EVALI are associated with vaping products containing THC or other cannabinoids as well as VEA. EVALI cases are more frequent in the United States than anywhere else. This is because VEA is not present in the United Kingdom and most European EC products as it is banned following the TPD directive. THC and other cannabinoids are unlikely to be present in licit U.K. EC products as cannabis is a controlled substance in the UK.[64]

### 7.2 | Other health complications from EC use

It is difficult to pinpoint what causes specific health complications from using ECs. Complex e-liquid mixtures result in a wide range of adverse health effects, from simple respiratory irritation to systemic diseases. A study from South Korea reported an increased risk for asthma and more severe asthma symptoms than the year before and this was directly related to regular e-cigarette use.[68] Several studies conducted on mice have found that additional ingredients such as flavourings are not the only cause of lung injury and that in fact, use of ECs containing PG, VG and nicotine contribute to lung toxicity, especially with daily long-term EC use. The symptoms in mice are comparable to those seen in humans such as airway inflammation.[67,69] In a 2016 study, when lung cells were exposed to 18 and 24 mg/ml nicotine e-liquids, there was an increase in immune response and cytokines. Below 18 mg/ml there were no differences in metabolic activity or cell viability.[40] Studies on epithelial lung cells have determined that certain flavourings such as acetoin, pentanedione and maltol impaired epithelial barrier function in human bronchial epithelial cells and exhibited proinflammatory response in lung cells.[70]

Nicotine EC aerosol exposure may well be associated with respiratory function impairment. The high levels of nicotine in some products increase risks for younger and nonhabitual users as there are fewer available sites for nicotine binding. Nicotine poisoning due to cigarettes is rare; however, ECs may pose an increased risk of nicotine toxicity due to higher nicotine concentrations in the e-liquids and cartridges as well as higher nicotine availability due to lack of combustion.[65] Additionally, the level of nicotine exposure from each puff is highly variable, there is variability in aerosolization and inconsistent nicotine delivery, many studies have found.[4,45,71,72] The FDA has claimed: 'There is too much variability in the amount of nicotine delivered per puff of any e-cigarette cartridge for them to be considered safe'. This, in addition to the issue of mislabelling means that it is almost impossible to know how much nicotine the user is inhaling on a regular basis. Moreover, a 2016 study compared liquid nicotine concentrations in e-liquids to plasma nicotine concentrations in accustomed EC smokers. The vapers were asked to take one puff every 30 s for a total of 10 puffs, wait an hour, then repeat the process once more. The average plasma concentration for the 36 mg/ml e-liquids was 30.2 μg/l.[73] This study revealed that nicotine rich e-liquids (36 mg/ml and above) caused a 'nicotine boost' in the plasma, much greater than what is seen when smoking traditional cigarettes under the same puffing conditions. This leads to not only a greater level of nicotine dependence but also nicotine toxicity.[73] As mentioned earlier, 115 μg/l of nicotine was found in a blood sample from a recently deceased person who had intentionally taken nicotine with the intent to harm themselves, although 5 mg/l of nicotine in the blood is generally considered to be the fatal level.[22]

## 8 | METHODS OF ANALYSIS FOR NICOTINE-CONTAINING E-LIQUIDS

Although ECs and nicotine are regulated in many countries, the production of these products may not be regulated at source, which would explain the prevalence of mislabelling and the presence of harmful or banned flavourings in e-liquids. Many EC products are imported and therefore, authorities need to carry out independent analysis to assess compliance with legislation. In addition to this, more often than not, the ingredients are partially labelled or not labelled at all. Lack of shelf life information is also a common issue.[53,74] There are methods that already exist for identifying and quantifying e-liquid components (Table 5); however, there is a need for a universal robust method that can test these products before they are released on the market to ensure they are safe enough for use. As the use of flavourings is abundant and varied, these methods need to consider possible interferences from these molecules. Ideally a rapid universal screening method should exist as a standard to quickly detect any potentially harmful ingredients in e-cigarette products, such as high throughput mass spectrometry (MS)-based methods for simple and rapid determination of target chemicals in complex matrices.[53]

Nicotine is the main ingredient in e-liquids that is regulated and e-liquid compositions are highly variable. Therefore, a sensitive, repeatable and simple method for determining the nicotine content should be a priority.[76] Many studies have mentioned discrepancies in nicotine levels between duplicates, using a variety of analytical methods.[27,48,49] A few determined that this was due to the e-liquid solutions being oily and viscous and hard to pipette accurately.[28,76] This leads to poor dispersion and lack of homogeneity when sampling e-liquids. There is room here for better sample preparation, by decreasing viscosity without modifying the nicotine content.

### 8.1 | Gas chromatography–based methods

It appears that gas chromatography (GC)-based methods are the preferred methods of analysis for nicotine. This is partly due to the fact that GC methods are well established and have been around for longer than liquid chromatography (LC) methods but also due to the fact that nicotine is relatively volatile and thermally stable, making GC an appropriate analytical method to quantify nicotine.[76]

**TABLE 5** Summary of analytical methods used to detect and quantify nicotine in e-liquids

| Reference | Method | Sample Preparation | Internal Standard | LOD | LOQ | Validated |
|---|---|---|---|---|---|---|
| [28] | UHPLC-DAD-UV | Diluted with 1 M ammonia to a 150-μg/ml concentration | Not given | 0.01–0.03 mg/ml | Not given | European Pharmacopeia validated method for nicotine (not validated for e-liquids) |
| [75] | GC-TSD | Diluted with 10-ml methanol | quinoline | Not given | 0.05 mg/ml | per ICH guideline Q2, 2005 Recovery 102%, Precision 17%, LOQ 0.05 mg/ml |
| [29] | LC-ESI-MS/MS | 0.05 ml diluted with Milli-Q water | Not given | Not given | Not given | Limited |
| [32] | HPLC | Information not given | Not given | 50 ng/ml | 10 μg/ml | Precision <1% |
| [33] | GC-MS | Dissolved in water, extraction with sodium hydroxide and toluene | Hexadecane | Not given | Not given | Recovery 101% |
| [46] | GC-FID | Information not given | n-heptadecane | Not given | 100 μg/ml | Yes, but details not provided |
| [35] | GC-NPD | Diluted with 10-ml methanol | quinoline | Not given | 0.05 mg/ml | Recovery 102% |
| [36] | GC-TSD | Diluted with 10 ml methanol | quinolone | 0.01 mg/ml | 0.05 g/ml | Recovery 102%, Precision 18% |
| [37] | GC-TSD | Diluted with 10-ml methanol | quinolone | Not given | 17.05 g/ml | Precision 17%, Recovery 102% |
| [38] | GC-MS/MS | NaOH, then 10 ml of methyl tert-butyl ether added | Nicotine-d3 nornicotine-d4 | 0.05 mg/g | Not given | Precision 3.1–3.4%, Accuracy 93.9%–97.9% |
| [39] | GC-MS | 25 μl diluted with 10-ml acetonitrile | naphthalene-d8 | 0.149 ng/ml | 0.452 ng/ml | Information not given |
| [30] | LC-MS/MS | Diluted with methanol | Nicotine-d4 | 0.3–20 ng/ml | 1–31.8 ng/ml | Precision <14.2%, recovery 75.8%–116.4% |
| [40] | GC-NPD | Diluted with methanol | Citation[35,75] | Citation[35,75] | Citation[35,75] | Citation[35,75] |
| [31] | HPLC-MS/MS | Diluted with 1:9 water-methanol | Nicotine-d4 | Not given | 10 ng/ml | Accuracy 96%–103%, interday precision 6–11% |
| [49] | GC-MS/FID | Diluted with methanol | Not given | 0.01 mg/ml | 0.1 mg/ml | Uncertainty ±20% |
| [13] | FT-ICR-MS<br>GC-MS | Protonation with HCl in MeOH and $H_2O$ = nicotinium ion directly analysable with FT-ICR-MS, toluene or ethyl acetate extraction | Benzene<br>Nicotinium-d3 | $1.62 \times 10^{-4}$ ng/ml (given as $1 \times 10^{-12}$ mol $L^{-1}$)<br>Not given | Not given<br>Not given | Accuracy within 2%<br>Accuracy 10 to 15% less than the spiked amount |
| [42] | HPLC | Diluted with mobile phase (acetonitrile-sodium hydrogen carbonate) | Not given | 0.07 μg/ml | 0.3 μg/ml | per ICH guideline Q2, 2005. Accuracy 100.3%–100.6%., Precision <1.1% RSD |
| [43] | GC-FID | Diluted 1:100 in methanol | quinoline | 0.36 ng/μl IDL 0.04 mg/g (28.5 ng) MDL | Not given | Recovery 101 ± 5.48 SD % |
| [19] | HPLC | Information not given | Not given | 0.01 mg/ml | 0.05 mg/ml | Information not given |
| [44] | GC-MS | Dissolved in pyridine, derivatization with BSTFA | Not given | Not given | Not given | Information not given |

(Continues)

**TABLE 5** (Continued)

| Reference | Method | Sample Preparation | Internal Standard | LOD | LOQ | Validated |
|---|---|---|---|---|---|---|
| [27] | GC-MS | Diluted 100:1 dichloromethane | n-heptadecane | Not given | 0.016 mg/ml (lowest calibrator) | Information not given |
| [45] | GC-MS | Diluted 1:300 methanol | caffeine | 1 μg/ml | 5 μg/ml | Recovery 99.3%, precision 5.4% RSD |
| [48] | HPLC | Diluted in distilled water | Not given | Not given | Not given | European Pharmacopeia method for nicotine (not validated for e-liquids) |
| [47] | LC-MS/MS | Diluted to 75 μg/ml with acetonitrile | nicotine-$d_4$ | Not given | 5 μg/ml (lowest calibrator) | Precision 1.02–9.90%, accuracy 0.200–5.88% error |

Analysing nicotine in e-liquids by GC requires extraction of nicotine using solvents such as ethyl acetate or toluene. Incomplete extraction will cause large measurement errors. One validation study found that GC-MS analysis resulted in quantities being measured that were 10 to 15% less than the spiked nicotine content, whereas Fourier-transform ion cyclotron resonance MS (FT-ICR-MS) analysis yielded results within a 2% difference from the spiked nicotine amount. The higher variation in the GC-MS analysis was reportedly due to loss of nicotine during the ethyl acetate extraction.[13] In most studies mentioned in this review, the detected nicotine was less than what was stated on the label. This could be due to mislabelling, but there is also a possibility this could be due to extraction (notably ethyl acetate) and analytical methods. Many nicotine related compounds are thermally unstable which may explain why many e-liquids quantified by GC methods have less nicotine than stated on the label.[72,77] One study, however, that used toluene to extract the nicotine from the e-liquid to remove any possible interferences from the sample being injected onto the GC had a 92.1% recovery.[33] Many of the GC-MS studies described the use of internal standards to aid in the quantification of nicotine which included nicotine-$_{d3}$,[38] nicotinium-$_{d3}$,[13] naphthalene-$_{d8}$,[39] hexadecane,[33] n-heptadecane,[27] and caffeine.[41] For GC-based techniques not involving mass spectrometry, quinoline[35,75] and quinolone[36,37] have been employed as well as n-heptadecane.[46]

Other e-liquid components can also be analysed via GC; however, this would require derivatization to ensure volatility. Derivatization is not always ideal when aiming for fast analysis and minimal sample preparation as well as performing a 'general unknown screening'.[78] Recently, GC has been coupled with ion mobility spectrometry (IMS) to allow rapid and sensitive analysis. Automated headspace sample preparation was used for simple and reproducible injection in a temperature-controlled environment. This method can be used in a nonlaboratory environment as it uses a portable device.[14]

## 8.2 | LC-based methods

LC-based methods solve the issues of thermally unstable nicotine-related compounds that are seen with GC based methods as LC only requires the compounds to be soluble, not volatilized. Certain LC methods such as HPLC-UV, however, are more prone to error due to flavouring compounds and colourings in e-liquids. These can cause major interferences. Many of the alkaloids present in tobacco are likely to coelute and UV detection will not be able to differentiate between the wavelengths.[72,77] A UHPLC-DAD method has been described for simultaneous quantification of nicotine and nicotine-related alkaloids in e-liquids. The sample preparation is minimal, using a dilute and shoot method. This is a suitable alternative to targeted MS/MS methods for routine quality control analyses.[79]

A few studies have used a targeted LC-MS/MS approach to identify and quantify nicotine and nicotine-related impurities. This is because this analytical method has been used successfully to determine nicotine in many biological fluids, however applicability to e-liquid matrices needs to be improved.[53,80] Matrix effects have been highlighted due to propylene glycol interfering with the ionization process. The use of suitable internal standards overcome variability of matrices and although many authors did not report details on the internal standards employed or indicate whether they performed studies into matrix effects, nicotine-$_{d4}$ was used as an internal standard in several of the LC-MS-based studies.[30,31,47] This indicates less variability in the choice of internal standards compared with GC-MS-based techniques. Furthermore, one group suggested diluting the e-liquid at least 1,000-fold prior to analysis based on the results of experiments into matrix effects[30] and other authors have used a dilution factor of up to 1:50,000.[31] However this could reduce the accuracy of nicotine quantification or dilute the nicotine to a level below the limit of quantification.[30]

## 8.3 | Other methods

GC-MS and LC-MS/MS are well-established and accurate methods for the analysis of nicotine in e-liquids.[73] However, other methods have been suggested to increase throughput, analyse the full contents of EC products using a combination of methods, or overcome difficulties with chromatographic separation and compounds/interferents with similar molecular weights.[53]

Differential ion mobility spectrometry (DMS) has been advocated as a high-throughput approach for the analysis of EC products. DMS separates ionized molecules based on their mobilities in the gas phase in the presence of an electric field. The mobility of an ion in these conditions depends on its size, charge and shape. DMS can be useful to resolve isobaric and isomeric compounds in complex mixtures. Although the method does not employ a preionization (chromatographic) separation step, matrix effects were not pronounced, ranging from −7% to 5% for nicotine, which could be attributed to high dilution factors used in sample preparation (typically 10,000-fold). Samples may even be directly injected thanks to the filtering action of DMS.[53] Another group has employed Direct Analysis in Real Time™ ionization source coupled to a time-of-flight mass spectrometer as a method to initially screen EC liquids.[31] Although the technique uses an open air ionization source, requires little or no sample preparation and provides information on the accurate mass of compounds, no information was provided on the dilution factors employed or potential matrix effects. Another group employed direct mass spectrometry using FT-ICR-MS and a 1/51 dilution of EC liquids but did not discuss matrix effects.[13]

A study in 2017 described a surface-enhanced Raman spectroscopy (SERS) method for the analysis of nicotine in e-liquids. This method used high dilution in the sample preparation which eliminated the effects of the viscous glycerine/glycerol medium and any flavouring agents. The nicotine concentrations analysed were several orders of magnitude above the working range of the SERS measurement, which allowed for accurate results even with high dilution factors.[74] The main drawback of this technique is that nicotine decomposes in air. Older e-liquid samples may show little to no signal intensity when analysed, therefore underestimating the nicotine content.

A recently developed method, boron doped diamond electrode (BDDE), has been described. This method is relatively simple as it only requires submerging the electrode into the e-liquid to measure nicotine content. Sample preparation is also straightforward, only requiring the e-liquid to be diluted; however, the electrode must be electrochemically activated for accurate operation. This specific electrode is able to maintain low background currents and show high repeatability, solving the issue of low repeatability commonly seen when analysing e-liquids with GC. The limit of detection for nicotine in one study was 0.01 mg/l. This method is simple, sensitive, accurate and rapid for determining nicotine content in samples with a complex matrix.[78]

As shown in Table 5, the LODs and LOQs vary by several magnitudes. This is mainly due to different studies looking at different nicotine concentrations as well as using different chromatographic methods and detection devices. For the studies looking at 'nicotine-free' products, the detection limits needed to be much lower in order to detect any possible nicotine. The analytical methods mentioned above all have their advantages and drawbacks; however, it depends what the focus of the analysis is. If the main focus is to quantify nicotine accurately, even in the presence of other ingredients, then a universal method should be used by manufacturing companies to ensure consistency and compliance with the law. However, other methods may be more suited for quantifying other ingredients such as flavourings. Even if several methods are needed to quality test a product before it reaches the market, these methods should at least be validated to ensure safe and high-quality products.

## 9 | LIMITATIONS

This study involved analysing other authors' data. The conclusions made are based on the assumption that their values are accurate. However, methods used for analysis were explored and potential areas for errors were identified and critically discussed. Not all products on the market have been assessed, nor have all studies on this topic been assessed. The mislabelling issue may lie with one or two main manufacturers that distribute to other brands. Every study used a different number of samples from various manufacturers and vendors, some used popular brands and others chose at random. This resulted in a high diversity of e-liquids but a lack in uniformity for this study. In addition to this, some studies only looked at one nicotine strength, whereas others looked at multiple nicotine strengths. It was assumed that the majority of the studies analysed here quantified nicotine in its free-base form; however, one study quantified free-base nicotine and nicotine salt in a regular e-liquid.[33] This made reported results difficult to compare across studies. The ±10% deviation from the labelled value has been adopted by AEMSA and BSI; however, many analytical methods allow for higher error, such as ±30% in toxicological validation studies. It is important to take this into account when e-liquids are being analysed.

## 10 | FURTHER STUDIES

To better understand the toxicological effects of all the components of e-liquids, more controlled toxicological studies should be performed. To date, most studies have analysed self-reported symptoms from vape smokers.[5,56,66,68] Blood sample analysis is less subjective than self-reporting symptoms. Monitoring blood samples for nicotine and other relevant analytes from the flavourings could give a better indication of what causes which symptom and how different concentrations correlate to effects. This could also highlight analyte metabolism and inter-analyte interactions. These studies would have to be conducted over varying time periods to assess short-term and long-term toxicity.

## 11 | CONCLUSIONS

E-cigarettes were initially introduced to reduce harm associated with smoking traditional cigarettes. They supposedly help reduce consumption and addiction; however, some e-liquids contain more nicotine than regular cigarettes ever have. What is most alarming is that many

of these products are targeted towards youths with fun and sweet flavourings and they are often unaware that ECs contain nicotine. There is clearly a lack of quality control when it comes to ECs and EC products, even in countries where these are heavily regulated. The possibility of online purchase, which is often more convenient for many people, makes it more difficult for users to purchase quality and trusted products. Personal online purchases can bypass the laws in a given country. Regulating e-cigarettes is complex as laws in each country vary. Products are manufactured, exported and imported worldwide. Mislabelling by between 10% and 20% was found to be a common occurrence, even though many countries have set a limit of ±10% deviation from the labelled value. The nicotine label values from the 23 studies were found to be statistically significantly different from the quantified nicotine values. This is dangerous not only when nicotine levels are above the stated value due to possible intoxication but also when nicotine concentrations are below the labelled value due to compensatory smoking behaviour and modifications of device power as this delivers unpredictable quantities of nicotine. ENDS need to be regulated, not only with their ingredients but also with electronics as modification can significantly enhance toxicity. Long-term health impacts from EC used are yet to be studied in depth; however, from preliminary trials on mice and on human lung cells in vitro, it is clear that high nicotine levels delivered via aerosol increase risk for nicotine poisoning and cause airway inflammation. Many other EC components, namely flavourings are CMR or contribute to EVALI. There are many suitable analytical methods available to analyse e-liquid components, however, each has different limits of detection and quantification as well as different recoveries, precision and accuracies. For EC products to be compliant with various laws, analytical methods need to be standardized and validated so that all products undergo rigorous and accurate testing. This area of study would benefit from further longitudinal toxicological studies on vapers to assess all aspects of EC product safety.

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## REFERENCES

1. Clapp PW, Pawlak EA, Lackey JT, et al. Flavored e-cigarette liquids and cinnamaldehyde impair respiratory innate immune cell function. *Am J Physiol Lung Cell Mol Physiol*. 2017;313(2):278-292. https://doi.org/10.1152/ajplung.00452.2016
2. Gilbert HA U.S. Patent No. 3,200,819. Washington, DC: U.S. Patent and Trademark Office 1965. https://patents.google.com/patent/US3200819A/en. Accessed October 18, 2020.
3. Lee YJ, Na CJ, Botao L, Kim KH, Son YS. Quantitative insights into major constituents contained in or released by electronic cigarettes: propylene glycol, vegetable glycerin, and nicotine. *Sci Total Environ*. 2020;703:1-9. https://doi.org/10.1016/j.scitotenv.2019.134567
4. Fearon IM, Eldridge AC, Gale N, McEwan M, Stiles MF, Round EK. Nicotine pharmacokinetics of electronic cigarettes: a review of the literature. *Regul Toxicol Pharmacol*. 2018;100:25-34. https://doi.org/10.1016/j.yrtph.2018.09.004
5. Walley SC, Wilson KM, Winickoff JP, Groner J. A public health crisis: electronic cigarettes, vape, and JUUL. *Pediatrics*. 2019;143(6):1-13, e20182741. https://doi.org/10.1542/peds.2018-2741
6. Bhatta DN, Glantz SA. Association of e-cigarette use with respiratory disease among adults: a longitudinal analysis. *Am J Prev Med*. 2020;58(2):182-190. https://doi.org/10.1016/j.amepre.2019.07.028
7. World Health Organization. E-cigarettes 2020. https://www.who.int/news-room/q-a-detail/e-cigarettes-how-risky-are-they. Accessed June 2 2020.
8. Allen JG, Flanigan SS, Leblanc M, et al. Flavoring chemicals in e-cigarettes: diacetyl, 2,3-pentanedione, and acetoin in a sample of 51 products, including fruit-, candy-, and cocktail-flavored e-cigarettes. *Environ Health Perspect*. 2016;124(6):733-739. https://doi.org/10.1289/ehp.1510185
9. Australian Government Department of Health. About e-cigarettes 2020. https://www.health.gov.au/health-topics/smoking-and-tobacco/about-smoking-and-tobacco/about-e-cigarettes. Accessed June 15, 2020.
10. TEDMED. What you should know about vaping and e-cigarettes [Video file] 2018. https://www.ted.com/talks/suchitra_krishnan_sarin_what_you_should_know_about_vaping_and_e_cigarettes?language=en. Accessed June 2 2020.
11. Zhu SH, Sun JY, Bonnevie E, et al. Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation. *Tob Control*. 2014;23(3):3-9. https://doi.org/10.1136/tobaccocontrol-2014-051670
12. Romberg AR, Miller Lo EJ, Cuccia AF, et al. Patterns of nicotine concentrations in electronic cigarettes sold in the united states, 2013-2018. *Drug Alcohol Depend*. 2019;203:1-7. https://doi.org/10.1016/j.drugalcdep.2019.05.029
13. Ogunwale MA, Chen Y, Theis WS, Nantz MH, Conklin DJ, Fu XA. A novel method of nicotine quantification in electronic cigarette liquids and aerosols. *Anal Methods*. 2017;29(9):4261-4266. https://doi.org/10.1039/C7AY00501F
14. Budzyńska E, Sielemann S, Puton J, Surminski A. Analysis of e-liquids for electronic cigarettes using GC-IMS/MS with headspace sampling. *Talanta*. 2020;209:1–7, 120594. https://doi.org/10.1016/j.talanta.2019.120594
15. Fagan P, Pokhrel P, Herzog TA, et al. Sugar and aldehyde content in flavored electronic cigarette liquids. *Nicotine Tob Res*. 2018;20(8):985-992. https://doi.org/10.1093/ntr/ntx234
16. Nedelman MC. New bill aims to cap nicotine levels in e-cigarettes 2019. https://edition.cnn.com/2019/10/07/health/vaping-nicotine-cap-krishnamoorthi-bn/index.html Accessed June 8 2020.
17. Public Health England. Vaping in England: 2020 evidence update summary 2020. https://www.gov.uk/government/publications/vaping-in-england-evidence-update-march-2020/vaping-in-england-2020-evidence-update-summary. Accessed June 15 2020.
18. Government of Canada. Vaping Product Regulations 2020. https://www.canada.ca/en/health-canada/services/smoking-tobacco/vaping/product-safety-regulation.html. Accessed June 15 2020.
19. Raymond BH, Collette-Merrill K, Harrison RG, Jarvis S, Rasmussen RJ. The nicotine content of a sample of e-cigarette liquid manufactured in the United States. *J Addict Med*. 2018;12(2):127-131. https://doi.org/10.1097/ADM.0000000000000376
20. Global Tobacco Control. Country laws regulating e-cigarettes 2020. https://www.globaltobaccocontrol.org/e-cigarette_policyscan. Accessed June 19 2020.
21. Walley SC, Jenssen BP. Electronic nicotine delivery systems. *Pediatrics*. 2015;136(5):1018-1026. https://doi.org/10.1542/peds.2015-3222
22. Clarke E. Clarke's analysis of drugs and poisons: nicotine 2020. https://www.medicinescomplete.com/#/content/clarke/c-d1e805183?hspl=nicotine. Accessed June 16 2020.

23. Benowitz NL, Hukkanen J, Jacob P. Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb Exp Pharmacol*. 2009;192:29-60. https://doi.org/10.1007/978-3-540-69248-5_2
24. O'Connell G, Pritchard JD, Prue C, et al. A randomised, open-label, cross-over clinical study to evaluate the pharmacokinetic profiles of cigarettes and e-cigarettes with nicotine salt formulations in US adult smokers. *Intern Emerg Med*. 2019;14(6):853-861. https://doi.org/10.1007/s11739-019-02025-3
25. VapeUK. The vape UK guide to nicotine salt 2020. https://vapeuk.co.uk/pages/nicotine-salt-vs-freebase-nicotine. Accessed June 18 2020.
26. Omaiye EE, McWhirter KJ, Luo W, Pankow JF, Talbot P. High-nicotine electronic cigarette products: Toxicity of JUUL fluids and aerosols correlates strongly with nicotine and some flavor chemical concentrations. *Chem Res Toxicol*. 2019;32(6):1058-1069. https://doi.org/10.1021/acs.chemrestox.8b00381
27. Srbinoska M, Kavrakovski Z, Rafajlovska V, Simonovska J. Determined and declared nicotine content in refill liquids for electronic cigarettes marketed in north Macedonia. *Arh Hig Rada Toksikol*. 2019;70(2):130-133. https://doi.org/10.2478/aiht-2019-70-3191
28. Etter J, Zäther E, Svensson S. Analysis of refill liquids for electronic cigarettes. *Addiction*. 2013;108(9):1671-1679. https://doi.org/10.1111/add.12235
29. Cameron JM, Howell DN, White JR, Andrenyak DM, Layton ME, Roll JM. Variable and potentially fatal amounts of nicotine in e-cigarette nicotine solutions. *Tob Control*. 2014;23(1):77-78. https://doi.org/10.1136/tobaccocontrol-2012-050604
30. Famele M, Palmisani J, Ferranti C, et al. Liquid chromatography with tandem mass spectrometry method for the determination of nicotine and minor tobacco alkaloids in electronic cigarette refill liquids and second-hand generated aerosol. *J Sep Sci*. 2016;40(5):1049-1056. https://doi.org/10.1002/jssc.201601076
31. Peace MR, Baird TR, Smith N, Wolf CE, Poklis JL, Poklis A. Concentration of nicotine and glycols in 27 electronic cigarette formulations. *J Anal Toxicol*. 2016;40(6):403-407. https://doi.org/10.1093/jat/bkw037
32. Davis B, Dang M, Kim J, Talbot P. Nicotine concentrations in electronic cigarette refill and do-it-yourself fluids. *Nicotine Tob Res*. 2015;17(2):134-141. https://doi.org/10.1093/ntr/ntu080
33. El-Hellani A, El-Hage R, Baalbaki R, Talih S, Shihadeh A, Saliba N. Quantification of free-base and protonated nicotine in electronic cigarette liquids and aerosol emissions. *Chem Res Toxicol*. 2015;28(8):1532-1537. https://doi.org/10.1021/acs.chemrestox.5b00107
34. Czoli CD, Goniewicz ML, Palumbo M, White CM, Hammond D. E-cigarette nicotine content and labelling practices in a restricted market: findings from Ontario, Canada. *Int J Drug Policy*. 2018;58:9-12. https://doi.org/10.1016/j.drugpo.2018.04.001
35. Goniewicz ML, Gupta R, Lee YH, et al. Nicotine levels in electronic cigarette refill solutions: a comparative analysis of products from the U.S., Korea, and Poland. *Int J Drug Policy*. 2015;26(6):583-588. https://doi.org/10.1016/j.drugpo.2015.01.020
36. Kim S, Goniewicz M, Yu S, Kim B, Gupta R. Variations in label information and nicotine levels in electronic cigarette refill liquids in South Korea: Regulation challenges. *Int J Environ Res Public Health*. 2015;12(5):4859-4868. https://doi.org/10.3390/ijerph120504859
37. Kosmider L, Sobczak A, Szołtysek-Bołdys I, et al. Assessment of nicotine concentration in electronic nicotine delivery system (ENDS) liquids and precision of dosing to aerosol. *Przegl Lek*. 2015;72(10):500-504.Retrieved from. https://pubmed.ncbi.nlm.nih.gov/26946554/
38. Lisko JG, Tran H, Stanfill SB, Blount BC, Watson CH. Chemical composition and evaluation of nicotine, tobacco alkaloids, pH, and selected flavors in e-cigarette cartridges and refill solutions. *Nicotine Tob Res*. 2015;17(10):1270-1278. https://doi.org/10.1093/ntr/ntu279
39. Crenshaw MD, Tefft ME, Buehler SS, Brinkman MC, Clark PI, Gordon SM. Determination of nicotine, glycerol, propylene glycol and water in electronic cigarette fluids using quantitative 1 H NMR: electronic cigarette fluids. *Magn Reson Chem*. 2016;54(11):901-904. https://doi.org/10.1002/mrc.4498
40. Leigh NJ, Lawton RI, Hershberger PA, Goniewicz ML. Flavourings significantly affect inhalation toxicity of aerosol generated from electronic nicotine delivery systems (ENDS). *Tob Control*. 2016;25(2):81-87. https://doi.org/10.1136/tobaccocontrol-2016-053205
41. Peace MR, Mulder HA, Baird TR, et al. Evaluation of nicotine and the components of e-liquids generated from e-cigarette aerosols. *J Anal Toxicol*. 2018;42(8):537-543. https://doi.org/10.1093/jat/bky056
42. Bansal M, Sharma M, Bullen C, Svirskis D. A stability indicating HPLC method to determine actual content and stability of nicotine within electronic cigarette liquids. *Int J Environ Res Public Health*. 2018;15(8):1-11. https://doi.org/10.3390/ijerph15081737
43. Dai J, Kim K, Szulejko JE, Jo S, Kwon K, Choi DW. Quantification of nicotine and major solvents in retail electronic cigarette fluids and vaped aerosols. *Microchem J*. 2018;140:262-268. https://doi.org/10.1016/j.microc.2018.04.028
44. Chivers E, Janka M, Franklin P, Mullins B, Larcombe A. Nicotine and other potentially harmful compounds in "nicotine-free" e-cigarette liquids in Australia. *Med J Aust*. 2019;210(3):127-128. https://doi.org/10.5694/mja2.12059
45. Zain SMSM, Khair SKJ, Mohamad N, Noh MFM, Radhakrishnan DA. Nicotine content in electronic cigarette refill solutions and its release in aerosols. *J Environ Prot*. 2019;10(10):1317-1332. https://doi.org/10.4236/jep.2019.1010078
46. Farsalinos KE, Gillman G, Melvin MS, et al. Nicotine levels and presence of selected tobacco-derived toxins in tobacco flavoured electronic cigarette refill liquids. *Int J Environ Res Public Health*. 2015;12(4):3439-3452.
47. Jackson R, Huskey M, Brown S. Labelling accuracy in low nicotine e-cigarette liquids from a sampling of US manufacturers. *Int J Pharm Pract*. 2020;28(3):290-294. https://doi.org/10.1111/ijpp.12596
48. Bennani I, Alami CM, El Karbane M, Cheikh A, Bouatia M. E-cigarette quality control: Impurity and nicotine level analysis in electronic cigarette refill liquids. *Sci World J*. 2020;2020:1-7. https://doi.org/10.1155/2020/3050189
49. Etter J, Bugey A. E-cigarette liquids: constancy of content across batches and accuracy of labeling. *Addict Behav*. 2017;73:137-143. https://doi.org/10.1016/j.addbeh.2017.05.012
50. Omaiye EE, Cordova I, Davis B, Talbot P. Counterfeit electronic cigarette products with mislabeled nicotine concentrations. *Tob Regul Sci*. 2017;3(3):347-357. https://doi.org/10.18001/TRS.3.3.10
51. Trtchounian A, Talbot P. Electronic nicotine delivery systems: is there a need for regulation? *Tob Control*. 2011;20:47-52. https://doi.org/10.1136/tc.2010.037259
52. Dawkins L, Cox S, Goniewicz M, et al. 'Real-world' compensatory behaviour with low nicotine concentration e-liquid: subjective effects and nicotine, acrolein and formaldehyde exposure. *Addiction*. 2018;113(10):1874-1882. https://doi.org/10.1111/add.14271
53. Regueiro J, Giri A, Wenzl T. Optimization of a differential ion mobility spectrometry–tandem mass spectrometry method for high-throughput analysis of nicotine and related compounds: application to electronic cigarette refill liquids. *Anal Chem*. 2016;88(12):6500-6508. https://doi.org/10.1021/acs.analchem.6b01241
54. Noël JC, Rainer D, Gstir R, Rainer M, Bonn G. Quantification of selected aroma compounds in e-cigarette products and toxicity evaluation in HUVEC/Tert2 cells. *Biomed Chromatogr*. 2019;34(3):1–12, e4761. https://doi.org/10.1002/bmc.4761
55. Behar RZ, Luo W, McWhirter KJ, Pankow JF, Talbot P. Analytical and toxicological evaluation of flavor chemicals in electronic cigarette refill fluids. *Sci Rep*. 2018;8(8288):1-11. https://doi.org/10.1038/s41598-018-25575-6
56. McConnell R, Barrington-Trimis JL, Wang K, et al. Electronic cigarette use and respiratory symptoms in adolescents. *Am J*

*Respir Crit Care Med*. 2017;195(8):1043-1049. https://doi.org/10.1164/rccm.201604-0804OC
57. Tierney PA, Karpinski CD, Brown JE, Luo W, Pankow JF. Flavour chemicals in electronic cigarette fluids. *Tob Control*. 2016;25(1):10-15. https://doi.org/10.1136/tobaccocontrol-2014-052175
58. Behar RZ, Luo W, Lin SC, et al. Distribution, quantification and toxicity of cinnamaldehyde in electronic cigarette refill fluids and aerosols. *Tob Control*. 2016;25(2):94-102. https://doi.org/10.1136/tobaccocontrol-2016-053224
59. Khlystov A, Samburova V. Flavoring compounds dominate toxic aldehyde production during e-cigarette vaping. *Environ Sci Technol*. 2016;50(23):13080-13085. https://doi.org/10.1021/acs.est.6b05145
60. Farsalinos KE, Lagoumintzis G. Toxicity classification of e-cigarette flavouring compounds based on European Union regulation: analysis of findings from a recent study. *Harm Reduct J*. 2019;16(48):1-8. https://doi.org/10.1186/s12954-019-0318-2
61. Kosmider L, Sobczak A, Prokopowicz A, et al. Cherry-flavoured electronic cigarettes expose users to the inhalation irritant, benzaldehyde. *Thorax*. 2016;71(4):376-377. https://doi.org/10.1136/thoraxjnl-2015-207895
62. Vas CA, Porter A, McAdam K. Acetoin is a precursor to diacetyl in e-cigarette liquids. *Food Chem Toxicol*. 2019;133:1–16, 110727. https://doi.org/10.1016/j.fct.2019.110727
63. Lisko JG, Lee GE, Kimbrell JB, Rybak ME, Valentin-Blasini L, Watson CH. Caffeine concentrations in coffee, tea, chocolate, and energy drink flavored e-liquids. *Nicotine Tob Res*. 2017;19(4):484-492. https://doi.org/10.1093/ntr/ntw192
64. Muthumalage T, Friedman MR, McGraw MD, Ginsberg G, Friedman AE, Rahman I. Chemical constituents involved in e-cigarette, or vaping product use-associated lung injury (EVALI). *Toxics (Basel)*. 2020;8(2):1–12, 25. https://doi.org/10.3390/toxics8020025
65. McCauley L, Markin C, Hosmer D. An unexpected consequence of electronic cigarette use. *Chest*. 2012;141(4):1110-1113. https://doi.org/10.1378/chest.11-1334
66. Callahan-Lyon P. Electronic cigarettes: human health effects. *Tob Control*. 2014;23(suppl 2):36-40. https://doi.org/10.1136/tobaccocontrol-2013-051470
67. Phillips B, Titz B, Kogel U, et al. Toxicity of the main electronic cigarette components, propylene glycol, glycerin, and nicotine, in sprague-dawley rats in a 90-day OECD inhalation study complemented by molecular endpoints. *Food Chem Toxicol*. 2017;109(1):315-332. https://doi.org/10.1016/j.fct.2017.09.001
68. Cho JH, Paik SY. Association between electronic cigarette use and asthma among high school students in South Korea. *Plos One*. 2016;11(3):1–13, e0151022. https://doi.org/10.1371/journal.pone.0151022
69. Lim HB, Kim SH. Inhalation of e-cigarette cartridge solution aggravates allergen-induced airway inflammation and hyper-responsiveness in mice. *Toxicological Res (Seoul)*. 2014;30(1):13-18. https://doi.org/10.5487/tr.2014.30.1.013
70. Chun V, Moshensky A, Shin J, Alexander LC. Chronic inhalation of unflavored nicotine and no-nicotine containing electronic cigarette vapor leads to impaired lung function. *Respirology*. 2018;197:A4452. https://doi.org/10.1111/resp.13419_31
71. Gerloff J, Sundar IK, Freter R, et al. Inflammatory response and barrier dysfunction by different e-cigarette flavoring chemicals identified by gas chromatography–mass spectrometry in e-liquids and e-vapors on human lung epithelial cells and fibroblasts. *Applied in vitro Toxicology*. 2017;3(1):28-40. https://doi.org/10.1089/aivt.2016.0030
72. Goniewicz ML, Hajek P, McRobbie H. Nicotine content of electronic cigarettes, its release in vapour and its consistency across batches: regulatory implications. *Addiction*. 2014;109(3):500-507. https://doi.org/10.1111/add.12410
73. Liu X, Joza P, Rickert B. Analysis of nicotine and nicotine-related compounds in electronic cigarette liquids and aerosols by liquid chromatography-tandem mass spectrometry. *Contrib Tobacco Res*. 2017;27(7):154-167. https://doi.org/10.1515/cttr-2017-0016
74. Ramôa CP, Hiler MM, Spindle T, et al. Electronic cigarette nicotine delivery can exceed that of combustible cigarettes: a preliminary report. *Tob Control*. 2016;25(1):6-9. https://doi.org/10.1136/tobaccocontrol-2015-052447
75. Goniewicz M, Kuma T, Gawron M, Knysak J, Kosmider L. Nicotine levels in electronic cigarettes. *Nicotine Tob Res*. 2013;15(1):158-166. https://doi.org/10.1093/ntr/nts103
76. Itoh N, Bell SEJ. High dilution surface-enhanced Raman spectroscopy for rapid determination of nicotine in e-liquids for electronic cigarettes. *Analyst*. 2017;142(6):994-998. https://doi.org/10.1039/c6an02286c
77. Flora JW, Wilkinson CT, Sink KM, McKinney DL, Miller JH. Nicotine-related impurities in e-cigarette cartridges and refill e-liquids. *J Liq Chromatogr Relat Technol*. 2016;39(17–18):821-829. https://doi.org/10.1080/10826076.2016.1266500
78. Kowalcze M, Jakubowska M. Voltammetric determination of nicotine in electronic cigarette liquids using a boron-doped diamond electrode (BDDE). *Diamond Relat Mater*. 2020;103:1–8, 107710. https://doi.org/10.1016/j.diamond.2020.107710
79. Barhdadi S, Desmedt B, Courselle P, Rogiers V, Vanhaecke T, Deconinck E. A simple dilute-and-shoot method for screening and simultaneous quantification of nicotine and alkaloid impurities in electronic cigarette refills (e-liquids) by UHPLC-DAD. *J Pharm Biomed Anal*. 2019;169:225-234. https://doi.org/10.1016/j.jpba.2019.03.002
80. Famele M, Ferranti C, Abenavoli C, Palleschi L, Mancinelli R, Draisci R. The chemical components of electronic cigarette cartridges and refill fluids: review of analytical methods. *Nicotine Tob Res*. 2014;17(3):271-279. https://doi.org/10.1093/ntr/ntu197

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